EXHIBIT A59

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Asbestos Exposure and Ovarian Fiber Burden

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Epidemiologic studies suggest increased risk of epithelial ovarian cancer in female asbestos workers and increased risk of malignancy in general in household contacts of asbestos workers. Ovaries were studied from 13 women with household contact with men with documented asbestos exposure and from 17 women undergoing incidental oophorectomy. Ovarian tissue was examined by analytic electron microscopy.

Significant asbestos fiber burdens were detected in 9 out of 13 women with household asbestos exposure (69.2%), and in 6 out of 17 women who gave no exposure history (35%). Three exposed women had asbestos counts over 1 million fibers per gram wet weight (23%), but only 1/17 women without an exposure history had a count that high (6%). Although asbestos has been documented as a contaminant of some older cosmetic talc preparations, the chrysotile and crocidolite types of asbestos we detected are more indicative of background and/or occupational exposure.

This study demonstrates that asbestos can reach the ovary. Although the number of subjects is small, asbestos appears to be present in ovarian tissue more frequently and in higher amounts in women with a documentable exposure history. © *1996 Wiley-Liss, Inc.*

KEY WORDS: asbestos, ovary, talc, environmental exposure

INTRODUCTION

Epidemiologic evidence suggests that there is an increased risk of ovarian carcinoma in female asbestos workers [Acheson et al., 1982; Graham and Graham, 1967; Keal, 1960; Newhouse et al., 1972, 1985; Wignall and Fox, 1982], and animal data show changes resembling early ovarian carcinoma after intraperitoneal injection of asbestos [Graham and Graham, 1967]. In addition, household contacts of asbestos workers have been shown to be at increased risk of developing asbestos-related disease [Joubert et al., 1991; Roggli and Longo, 1991], so female household contacts of asbestos workers may also be at risk of ovarian exposure to asbestos. There is literature that supports that perineal talc exposure increases the risk of ovarian carcinoma. However, some of these data have been clouded by the fact that cosmetic talc was often contaminated with asbestos in the past, particularly before 1976 [Cramer et al., 1982]. Particulate matter can reach the female peritoneal cavity via the transvaginal route [Egli and Newton, 1961; Henderson et al., 1986; Joubert et al., 1991]. A woman exposed to her husband's occupationally contaminated laundry may have asbestos enter the peritoneal cavity by passive transfer, or even by sexual relations. The purpose of this study was to determine whether women exposed to asbestos have a high asbestos fiber burden in their ovaries.

MATERIALS AND METHODS

Eligible women were contacted by postcard with the assistance of a law firm specializing in asbestos-related claims. Women with household contact to asbestos, as documented by interview, and who had themselves previously undergone ovarian surgery, were invited to participate. No women with direct occupational exposure responded.

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TABLE I. Demographics and Pathologic Findings Among 8 Patients With Household Contacts Evaluated for Asbestos-Related Disease Oophorectomized Between 1973–1994, and 5 Oophorectomized Patients With a History of Asbestos Exposure From the Columbia Presbyterian Medical Center Benign Neoplasm Study, 1992–1993

Subject	Reason for surgery	Asbestos-fibers per gram wet weight	Limits of detection	Asbestos exposure
1	Papillary serous cystadenocarcinoma of ovary	490,813 chrysotile: crocidolite 1:2	40,901	Husband—pipefitter with asbestos
2	Mucinous cystadeno-carcinoma of ovary	below detectible limits	26,267	Father—died of mesothelioma; husband—asbestosis (both insulators)
3	Endometrial adenocarcinoma	1,227,031 chrysotile: crocidolite 1:1	15,338	Husband—asbestosis, carpenter in a factory
4	Atypical hyperplasia of endometrium	74,167 chrysotile	18,542	Husband—insulator, died of lung cancer
5	Endometriosis of ovary	328,913 chrysotile	41,114	Father and aunt—worked in asbestos plant; father—died of lung cancer, aunt of asbestosis
6	Leiomyoma uteri	3,438,636 chrysotile	42,983	Father—asbestosis, asbestos and insulation worker
7	Serous cystadenoma of ovary, fibroma of ovary	below detectible limits	42,983	Husband—insulation worker
8	Endometrial adenocarcinoma	1,513,00 chrysotile: tremolite 4:1	37,825	Husband—died of asbestosis;[a] worked as carpenter and with concrete
9[a]	Cystadenofibroma of ovary	49,081 chrysotile: crocidolite 1:1	24,541	2 brothers—construction workers
10[a]	Benign epithelial cyst of ovary	below detectible limits	37,825	Father—shipyard worker and school engineer
11[a]	Serous cystadenoma of ovary	298,618 chrysotile	24,885	Household member—shipyard worker × 4 years
12[a]	Cystadenofibroma of ovary	788,020 crocidolite	157,604	Father—shipyard worker
13[a]	Cystadenofibroma of ovary	below detectible limits	42,983	Household contact —construction/insulation × 3 years

[a]Subjects from Columbia Presbyterian Medical Center Benign Neoplasm Study.

Women with both benign and malignant disease responded. Women undergoing oophorectomy for benign ovarian neoplasms at Columbia Presbyterian Medical Center who were interviewed in depth for another study were available as controls and were included after ascertaining asbestos exposure history and availability of nonneoplastic ovarian tissue for analysis. These women were chosen for the availability of interviews as well as tissue. Five of these women were found to have sustained asbestos exposure. There were 13 exposed subjects and 17 women who gave no history of exposure. Tissue from two stillborn ovaries was also analyzed. Tissue blocks of benign adjacent or contralateral ovarian tissue as available were obtained, and analytic electron microscopy was performed according to the subsequent protocol. Hematoxylin and eosin stained sections of analyzed tissue were examined. There was no evidence of response to asbestos such as foreign body giant cell reactions or fibrosis in the tissue. Ovarian tissue does not undergo fibrosis as does lung.

Analytic Electron Microscopy Protocol

Ovarian tissue in blocks was deparaffinized, rehydrated, blotted dry, and weighed. Digestion with 5% KOH was performed at 70°C for 2–4 hr. After complete digestion, the tissue was centrifuged at 12,000 rpm for 20 min. The KOH was removed, leaving a pellet to which approximately 20 ml of distilled water was added. The pellet was resuspended by using a microultrasonic cell disrupter at 50 watts for 5 sec. Centrifugation, distilled water wash, and

TABLE II. Demographics and Pathologic Findings Among 17 Oophorectomized Patients With No History of Asbestos Exposure—Columbia Presbyterian Medical Center Benign Neoplasm Study, 1992–1993

Control	Reason for surgery	Asbestos fibers per gram wet weight	Limits of detection	Exposure history
11 subjects	4 serous cystadenomas/simple cyst 3 benign cystic teratoma/struma ovarii 2 endometrioma/endometriosis 1 fibrothecoma 1 mucinous cystadenoma	Below detectable limits	None greater than 27,267	None
1	Endometriosis, benign cystic teratoma	525,871 chrysotile: crocidolite 1:2	17,529	None
2	Endometrioma of ovary	109,069 chrysotile: crocidolite 1:1	6,817	None
3	Benign cystic teratoma of ovary	33,849 chrysotile	8,462	None
4	Endometrioma of ovary	147,244 chrysotile: crocidolite 1:2	12,270	None
5	Serous cystadenoma of ovary	98,163 crocidolite	12,270	None
6	Benign cystic teratoma of ovary	2,181,388 chrysotile	27,267	None

microultrasonic cell disrupter were repeated 3 times. The distilled water was removed and the pellet was resuspended in 5–10 ml of distilled water. Ten-μl drops of the final suspension were placed on nickel Formvar and carbon-coated locator grids and air dried. Transmission electron microscopy to identify fibers, and their size was performed. The identity of the fibers was determined by energy-dispersive spectroscopy and confirmed by electron diffraction (SAED). Grids were viewed at both 10,000 and 19,000 diameters. All fibers observed were counted.

Routinely, as they are opened or distilled water at each filtering, all solutions are checked for detectable limits of asbestos fibers. All places where asbestos could have contaminated the specimen, such as paraffin, are also controlled for paraffin blocks from each different source. All solutions are checked by passing the fluids through a 0.1-μm Nucleopore filter to maximize the efficiency of detecting and counting fibers present in these solutions and materials. The solutions that are routinely tested are distilled water, KOH, and xylene. If detectable levels of asbestos fibers exist in the solutions used to initially fix and process the tissue because they came from different hospitals, at which it was not possible to test these solutions directly, they would be detected in the paraffin controls. We have yet to identify detectable levels in any of the solutions or paraffin.

RESULTS

A summary of the results can be seen in Tables I and II. Nine of the 13 women exposed to asbestos had asbestos in their ovarian tissue (69.23%), with 3 (23%) of them having counts over 1 million fibers per gram of wet weight. Among the controls, 6/17 women had detectable asbestos in their ovaries (35%), with only 1 (6%) patient with a count over 1 million fibers per gram wet weight. In addition, talc was detected in 11/13 exposed women (85%) and in all 17 controls (100%). No asbestos or talc was detected in the stillborn material.

All fibers were counted and analyzed for type and size. The results of that analysis are summarized in Table III. In general, the fibers were relatively small with regard to length and narrow in diameter. However, the great majority of fibers were greater than 3 μm with a minimum aspect ratio of 10. Except for one case, in which tremolite was observed, the fibers were either chrysotile or crocidolite, or both.

DISCUSSION

Epithelial ovarian carcinoma is a major cause of female mortality [Greene et al., 1984]. Epithelial ovarian cancer

TABLE III. Asbestos Fibers in Ovarian Tissues: Type, Number, and Dimensions

Subject	No. of fibers	Fiber type	<3 μm long	3–10 μm long	>10 μm long	<0.1-μm diameter	0.1–0.2-μm diameter	>0.2-μm diameter
1[a]	4	Chrysotile	1	2	1	4	—	—
	8	Crocidolite	1	7	—	4	4	—
3[a]	40	Chrysotile	2	28	10	35	5	—
	40	Crocidolite	3	31	6	30	10	—
4[a]	4	Chrysotile	—	3	1	4	—	—
5[a]	8	Chrysotile	1	6	1	7	1	—
6[a]	80	Chrysotile	5	62	13	71	9	—
8[a]	32	Chrysotile	2	22	8	22	10	—
	8	Tremolite	1	7	—	—	6	2
9[a]	1	Chrysotile	—	1	—	1	—	—
	1	Crocidolite	—	1	—	1	—	—
11[a]	12	Chrysotile	1	9	2	8	4	—
12[a]	20	Crocidolite	2	14	4	12	8	—
1[b]	10	Chrysotile	1	8	1	4	6	—
	20	Crocidolite	2	18	—	17	3	—
2[b]	8	Chrysotile	—	7	1	5	3	—
	8	Crocidolite	1	7	—	6	2	—
3[b]	4	Chrysotile	—	4	—	3	1	—
4[b]	4	Chrysotile	—	4	—	3	1	—
	8	Crocidolite	1	7	—	7	1	—
5[b]	8	Crocidolite	—	8	—	6	2	—
6[b]	80	Chrysotile	7	58	15	68	12	—

[a]From Table I.
[b]From Table II.

develops from the surface epithelium of the ovary, which is embryologically derived from the same tissue as the mesothelium of the abdominal cavity, the celomic epithelium [Falkson, 1985]. Thus, ovarian carcinoma and malignant mesothelioma of the peritoneal cavity are believed by some to be related neoplasms [Parmely and Woodruff, 1974]. Asbestos causes malignant mesothelioma, and there is evidence to support it as an etiology in ovarian carcinoma as well [Acheson et al., 1982; Falkson, 1985; Graham and Graham, 1967; Keal, 1960; Newhouse, 1979; Newhouse et al., 1972, 1985; Whittemore et al., 1988; Wignall and Fox, 1982].

Intraperitoneal injection of tremolite asbestos into guinea pigs and rabbits was shown to cause epithelial changes in their ovaries similar to those seen in early ovarian cancer [Graham and Graham, 1967]. These investigators also found birefringent crystalline material near these epithelial changes, but no further attempt was made to identify the material. No such material was found in controls. Asbestos fibers have been shown to be cytotoxic to Chinese hamster ovary (CHO) cells, an epithelioid cell culture line [Neugut et al., 1978].

Several investigators have cited an increased mortality from ovarian cancer in female asbestos workers exposed as gas mask assemblers or other factory workers [Acheson et al., 1982; Newhouse, 1979; Newhouse et al., 1972, 1985; Wignall and Fox, 1982]. In addition, it is known that household contacts of asbestos exposed workers are also at increased risk of developing malignant disease in general [Joubert et al., 1991; Roggli and Longo, 1991]. In a study of 52 histologically confirmed malignant mesotheliomas in women, most with no occupational exposure of their own, a significant number were found to have husbands or fathers who worked in an asbestos-related industry [Vianna and Polan, 1978], and the findings suggested indirect exposure to a husband as the most important factor.

The fact that exposure to a husband is more significant than exposure to a father suggests a possible role for sexual contact as a transporting vector for asbestos fibers. Household exposure has been related to the asbestos dust on the workers' clothing, with risk to those who launder the clothing [Joubert et al., 1991]. While this may be the exposure source in wives as well as in daughters, it is possible that sexual contact with a male contaminated with asbestos fibers introduces those fibers into the vagina of his partner, where they can reach the peritoneal cavity. There is evi-

dence of transport of particulate matter into the female peritoneum by the transvaginal route, in both human and animal studies [Egli and Newton, 1961; Henderson et al., 1986; Venter and Iturralde, 1979]. Whittemore et al. [1988] suggested that vaginal exposure to particulate matter such as asbestos and talc was a potential risk factor for intraperitoneal ovarian exposure. Her conclusion was based on finding that in talc-exposed women, a previous history of hysterectomy or tubal ligation, which blocks peritoneal access, was protective against ovarian cancer.

Talc has also been implicated as a possible etiologic agent in ovarian cancer [Harlow et al., 1989, 1992], and this is related to the asbestos problem in several ways. Aside from the chemical similarities between the two, many cosmetic talcs contained significant amounts of asbestos, particularly prior to 1976 [Cramer et al., 1982]. The significance of the detection of talc in the majority of the exposed women and in all women giving no exposure history is unclear, and further studies are under way to further elucidate this association.

CONCLUSIONS

In our study, the women with a positive exposure history had asbestos detected in their ovaries more frequently, and in higher counts. None of the exposed subjects in this study was directly occupationally exposed, but all were passively exposed to a household contact. It is unclear why so many of the women giving no exposure history did have detectable asbestos in their ovaries, although it is known that there is a background level of asbestos in the lung tissue of nonexposed individuals. All our available control patients were selected from a group of extensively interviewed women with benign ovarian neoplasms. Further studies are aimed at women with no ovarian pathology. The significance of the finding of asbestos in ovaries requires further investigation.

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